Cancer Biology

Complex multicellular organisms contain two basic classes of cells: post-mitotic and mitotic cells. Post-mitotic cells cannot divide, although they may function throughout adult life. Examples of post-mitotic cells are mature neurons, adipocytes (fat cells), and mature muscle cells. Mitotic cells, by contrast, retain the ability to divide throughout life. Mitotic cells may divide continually, or only when there is a need for cell replacement or tissue repair. Mitotic cells include the differentiated (specialized) cells of epithelial tissues, such as skin, liver, colon, lung, breast and prostate. They also include certain cells of the immune system, such as T and B lymphocytes, and cells that support epithelial and post-mitotic cells, such as fibroblasts and glia. Cancers never arise from post-mitotic cells. Rather, cancers arise from mitotic cells.

Cancers in children and young adults tend to be due to defects in development or tissue maturation. Early life cancers include certain cancers of the developing immune system (leukemias), retinoblastoma (cancer of the developing retina) and osteosarcoma (bone cancer). Early life cancers tend to arise from precursors to post-mitotic cells (for example, neuroblastoma, tumor composed of precursors to neurons), or mitotic cells that support epithelial or post-mitotic cells (for example, fibrosarcoma or glioblastoma, tumors composed of fibroblasts and glial cells, respectively).

By contrast, most cancers that arise during middle and old age derive from epithelial cells, and, to a lesser extent, the immune system. Epithelial tissues such as skin, stomach and colon are composed of cells that are constantly sloughed off and replaced, and therefore contain cells that proliferate constantly throughout life. Other tissues show periodic or relatively slow proliferation. Examples include the breast, where epithelial cells proliferate with each estrus

cycle, and the mature immune system, where cells proliferate in response to specific antigens. Tissues that show constant or periodic cell proliferation are particularly prone to developing tumors later in life. Tissues in which cells divide relatively infrequently are much less susceptible to developing into cancer with age. Examples include liver and kidney, as well as cells that support and direct the functions of epithelial and post-mitotic cells such as fibroblasts and glia. However, chronic injury, toxicity or infection can greatly increase tumor incidence in these tissues.

What is cancer?

Cancer is the proliferation (used here interchangeably with growth) of malignant cells – cells that grow inappropriately, disrupt normal tissue structure and function, and, frequently, can survive in the blood stream and proliferate at distal sites. Cancer, if unchecked, can kill the organism.

Loss of growth control *per se* does not define a cell as malignant. Malignant cells have additional properties, specifically an ability to migrate and infiltrate the surrounding normal tissue (invasiveness), and induce a blood supply to feed the growing tumor (angiogenesis). Cancer cells invariably lose their differentiated properties, and eventually acquire the ability to colonize and invade distal tissues (metastasis).

Loss of growth control. All cancers are characterized by abnormal cell growth, or hyperproliferation. This property is termed neoplasia. Neoplasias may be benign (not invasive or metastatic) or malignant (invasive and frequently metastatic). Benign tumors are rarely fatal because their growth, however abundant, is rarely unlimited (and, additionally, they are rarely

invasive or metastatic). Malignant tumors, by contrast, tend to grow progressively, even if slowly.

What governs the controlled growth of normal cells? And what goes awry to cause the abnormal but limited growth of benign tumor cells and unlimited growth of malignant cells?

Normal cell proliferation is controlled by an exquisite balance between processes that stimulate growth and those that inhibit growth. When this balance is upset, abnormal growth occurs. Abnormal growth is generally caused by an increase in growth stimulatory processes, as well as a decrease in growth inhibitory processes.

External signals. Many growth stimulatory and inhibitory processes are triggered by signals that originate outside the cell (the cellular microenvironment). These external signals can be delivered by small diffusible molecules, such as growth factors or circulating hormones, or by large molecules, such as components of the extracellular matrix or basement membrane. They can also be delivered by adjacent or nearby cells. For example, fibroblasts, which produce the collagen-rich matrix (stroma) that underlies most epithelial layers, signal and instruct the epithelial cells. External growth regulatory molecules generally act by binding and altering transmembrane cell surface receptors. The altered receptors then associate with or modify intracellular proteins at the underside of the cell surface. These molecules, in turn, produce small diffusible chemical signals that, by interacting with or modifying yet other intracellular proteins, eventually send a signal to the cell nucleus. In the nucleus, specific genes that stimulate or inhibit progression through the cell cycle are then switched on or off.

Cellular senescence. Cell proliferation can also be governed by signals that originate within cells. A prime example is cellular senescence, an intrinsic program that causes mitotic cells to irreversibly withdraw from the cell cycle.

Most cells from adult organisms cannot divide indefinitely owing to a process termed replicative senescence. In humans (and certain other species), replicative senescence occurs because cells lose a small amount of DNA from the chromosome ends (the telomeres) after each round of DNA replication, and cells irreversibly arrest growth when they acquire one or more critically short telomere. Such cells are said to be replicatively senescent. Mitotic cells enter a state that closely resembles replicative senescence when they experience sublethal DNA damage, supraphysiological growth signals, or expression of certain oncogenes (genes that promote neoplastic growth, discussed below). Replicative senescence, then, is a special example of a more general process termed cellular senescence. Short telomeres, DNA damage, over-exuberant growth signals, and oncogenes all have the potential to change a normal cell into a pre-cancerous or cancer cell. Thus, cellular senescence, or the senescence response, suppresses tumorigenesis by preventing the growth of cells at risk for malignant transformation.

Cellular senescence appears to be a major barrier that cells must overcome in order to become malignant. Several lines of evidence support this idea. First, most, if not all, malignant tumors contain cells that have overcome cellular senescence. (Many tumor cells accomplish this by expressing telomerase, the enzyme that replenishes the telomeric DNA that is lost during DNA replication. Telomerase is expressed by the germ line and early embryonic cells, but is repressed in most adult cells.) Second, some oncogenes act by allowing cells to ignore senescence-inducing signals. Cells that express such oncogenes continue to proliferate despite short telomeres, DNA damage, or other potentially oncogenic conditions. Third, cellular

senescence is controlled by the p53 and pRB tumor suppressor proteins (discussed below), the two most commonly mutated tumor suppressors in human cancers. Finally, mutant mice have been generated in which cells fail to respond to senescence signals. Such animals invariably die at an early age of cancer.

Cellular senescence is also thought to contribute to aging. At first glance, this idea may seem at odds with its tumor suppressive function. It is consistent, however, with the theory of evolutionary antagonistic pleiotropy. This theory predicts that, because the force of natural selection declines with increasing age, some traits that were selected to optimize fitness during development or young adulthood can have unselected deleterious effects in aged organisms. In the case of cellular senescence, the irreversible growth arrest may be the selected trait, which prevents the proliferation of cells at risk for malignant transformation. Other features of the senescent phenotype -- resistance to programmed cell death and changes in cell function -- may be unselected and deleterious.

Some senescent cells are resistant to programmed cell death (apoptosis), and all senescent cells display changes in cell function. The functional changes can be striking. For example, senescent fibroblasts secrete degradative enzymes, inflammatory cytokines and growth factors. Thus, senescent fibroblasts can create a microenvironment that resembles chronic wounding, which can disrupt tissue structure and/or function as senescent cells accumulate. Senescent cells appear to be relatively rare in young tissues, but more common in old tissues. As discussed below, their ability to disrupt tissue structure may contribute to aging, as well as the rise in late life cancers.

Apoptosis. Most tissues achieve and maintain proper size by a balance between cell proliferation and death. All cells are capable of an orderly suicide process termed

programmed cell death or apoptosis. Apoptosis is important during embryogenesis, where it can rid the fetus of excess or damaged cells, or cells that fail to receive signals needed for proper function. Apoptosis is also important in adults, where is helps maintain the size of cell populations or tissues. Equally important, apoptosis removes damaged cells from adult tissues, and thus is another tumor suppressive mechanism.

Many tumor cells develop defects in the control of apoptosis. Consequently, many tumor cells survive under circumstances that would cause their normal counterparts to die. There is limited but compelling evidence that, in at least some tissues, apoptosis declines with increasing age. This decline may also contribute to the increase in age-related cancer.

Loss of differentiation. Another feature of cancer is abnormal differentiation, or anaplasia.

All cells contain the same DNA, and hence the same genome (30,000-50,000 genes, in humans). However, each cell expresses (transcribe into RNA and translate into protein) only 10-20% of their genome. The selective expression of genes is termed differentiation, and is responsible for the characteristics that distinguish different cell types from each other.

Differentiation begins early in embryogenesis. By adulthood, most tissues have matured and function much as they will throughout life. Nonetheless, differentiation is an ongoing process in tissues that rely on stem cells for renewal or repair. Stem cells are mitotic cells that, upon division, either produce another stem cell or produce a differentiated cell. In the skin, for example, stem cells renew themselves but more frequently give rise to basal keratinocytes. Basal keratinocytes, in turn, divide and differentiate into cells that form the upper layers of the epidermis. Throughout life, basal cells divide and progressively differentiate into upper

keratinocytes, including the outermost post-mitotic cells that are eventually sloughed off. The stem cells divide relatively infrequently, but enough to maintain the pool of basal cells. Differentiation not only determines which specialized proteins are made by a cell, but also whether cells are mitotic or post-mitotic, whether a cell proliferates or dies, and whether and how a cell migrates or communicates with other cells.

Because differentiation integrates cell growth and death with function, it is not surprising that tumors invariably show signs of abnormal differentiation. Tumor cells are generally less differentiated than surrounding normal cells, and the most aggressive tumors tend to be the least differentiated. Some cancers may arise directly from the least differentiated cells in a tissue, or the stem cells. In other cases, a cancer cell may acquire an abnormal pattern of gene expression, leading to a less differentiated state.

Childhood and young adult cancers tend to be poorly differentiated, generally arising from precursor or stem cells. Cancers of old adults, by contrast, include both poorly differentiated and relatively well-differentiated tumors.

Angiogenesis, invasion and metastasis. Malignant tumors acquire the ability to migrate and invade the surrounding normal tissue (invasiveness). They also stimulate the formation of blood vessels (angiogenesis), which provide the growing tumor with nutrients. The most malignant tumors acquire the ability to survive in the blood stream and colonize distal tissues (metastasis). These abilities are also characteristic of fetal cells. Hence, the anaplasia of tumor cells is often responsible for their invasive, metastatic and angiogenic properties.

Cancer cells become invasive when they secrete enzymes that degrade the extracellular matrix and stroma, and angiogenic when they secrete cytokines that attract endothelial and other

cells needed for blood vessel formation. Many tumor cells also secrete factors that cause stromal fibroblasts to secrete degradative enzymes and endothelial attractants. Although cancer incidence increases exponentially with age, tumors in very old individuals tend to be less aggressive than tumors in middle aged adults. This age-dependent difference may reflect the response of the surrounding host cells. Indeed, some tumors are less vascularized in older hosts because their endothelial cells respond much less well to tumor-derived angiogenic factors.

Metastasis requires that solid tumor cells acquire the ability to survive in the hostile environments of the blood stream and a foreign (ectopic) tissue. Most normal cells (and even most preneoplastic or benign tumor cells) undergo apoptosis when placed in a foreign environment. Metastatic tumor cells either fail to sense environmental cues that normally cause cell death, or fail to execute the apoptotic program. In addition, metastatic tumor cells frequently express cell surface proteins that allow them to adhere to and infiltrate an ectopic site.

Genomic instability. Another hallmark of malignant tumors is genomic instability. A prime cause of cancer is the accumulation of mutations (discussed below). Most cancers develop from preneoplastic cells – cells that have acquired one or a few mutations that confer a growth or survival advantage. Preneoplastic cells are not malignant, but are predisposed to malignant transformation upon acquiring additional mutations. During the 80's and 90's, cancers were thought to develop because cells successively acquired a discrete number of mutations, generally half a dozen or so, depending on the tissue. However, recent findings show that most tumors harbor many mutations, often exceeding several dozen.

Spontaneous mutation at any locus tends to occur once every hundred thousand or so cell divisions. How, then, do tumors acquire dozens of mutations during progression to malignancy?

Most cancer cells eventually acquire a mutation in one or more genes that ensure genomic stability -- the remarkable fidelity with which nuclear DNA and chromosome organization are maintained. These proteins, often referred to as guardians of the genome, include the p53 tumor suppressor, which halts the cell cycle when DNA is damaged. They also include proteins that regulate chromosome segregation during mitosis, and participate in DNA repair. As discussed below, some of these genes can be considered longevity assurance genes – genes that, when lost or mutated, shorten life span and accelerate certain age-related pathologies.

Once a cell loses the activity of one or more guardians of the genome, the genome becomes unstable and mutations occur much more frequently. A high mutation rate allows cancer cells to evolve rapidly. Thus, the genomic instability of cancer cells allows rapid selection for cells that have ever more aggressive and malignant properties.

What causes cancer?

Cancer is caused by a combination of genetic and epigenetic factors.

Genetic factors include mutations, both germ line and somatic, and polymorphisms. Mutations substantially change the expression levels, activities or functions of encoded proteins. Many childhood cancers, but a minority of adult cancers, are caused by germ line mutations – inherited mutations that are present in the DNA of every cell in the organism. By contrast, most adult cancers are caused by somatic (non-germ line) mutations – mutations acquired by a somatic cell during development, maturation or, most commonly, adulthood. On the other hand, polymorphisms -- minor sequence variations that only subtly change protein expression, activity or function -- rarely cause disease, but can predispose individuals to develop diseases, including cancer. Individuals within a species differ, despite a common genome, largely because most

genes exist in multiple forms (polymorphisms) that differ by one or a few nucleotides. Perhaps the best known example is the ApoE4 variant of the ApoE gene, which predisposes individuals to Alzheimer's disease. Polymorphisms that are thought to predispose individuals to developing cancer occur in genes that control cell growth, differentiation or apoptosis, or enzymes that metabolize carcinogens or anti-carcinogen.

Epigenetic factors that cause cancer include reversible changes to DNA, such as DNA methylation or posttranslational modification of DNA associated proteins, such as histones. Such changes generally alter the compaction of chromatin (DNA plus associated proteins), which can have profound effects on gene expression. In addition, the tissue structure and hormonal milieu in which a potential cancer cell exists can strongly influence whether and to what extent it progresses to form a malignant tumor. The influence of tissue structure may be particularly important for the development of late life cancers, as will be discussed further below.

Cancer-causing mutations. Cancer-causing mutations range from single base changes (point mutations) to gross rearrangements, losses and/or amplifications of large regions of chromosomes. The latter mutations generally occur when cancer cells develop genomic instability. What are the targets of cancer-causing mutations? Given the features of cancer cells – abnormal growth, resistance to apoptosis, loss of differentiation and genomic instability – there are many genes that, when mutated, can contribute to malignant transformation. In general, these genes fall into either of two categories: oncogenes and tumor suppressor genes.

Oncogenes. Oncogenes are mutant forms of normal cellular genes termed proto-oncogenes. Proto-oncogenes generally encode proteins (proto-oncoproteins) that stimulate cell proliferation, regulate apoptosis, or restrain differentiation. In normal cells, the activities of

proto-oncoproteins are tightly regulated. Mutations that convert a protooncogene into an oncogene often render the encoded protein resistant to normal regulatory constraints, or cause the protein to be overexpressed. Mutations that convert proto-oncogenes to oncogenes generally result in a gain-of-function – that is, the mutation confers new or supraphysiological properties to the protein. Such mutations are dominant because only one of the two gene copies need be mutant in order for the mutation to exert its effects.

What types of proteins are encoded by proto-oncogenes, and what types of mutations convert or activate them to become oncogenes? Proto-oncoproteins include growth factors, growth factor receptors and their signal transduction proteins, growth stimulatory transcription factors, and anti-apoptotic proteins. Activating mutations range from subtle point mutations to gross chromosome rearrangements that create novel chimeric proteins.

An example of a simple activating point mutation is illustrated by members of the *RAS* proto-oncogene family. RAS proteins bind GTP in response to growth factor receptor occupancy, whereupon they transduce a growth stimulatory signal. Shortly thereafter, the intrinsic GTPase activity of the RAS proteins converts the bound GTP to GDP, thereby attenuating the growth signal. Oncogenic mutations in *RAS* tend to be point mutations that abolish GTPase activity, but not GTP binding, thereby causing a constitutively active growth signal. The other end of the spectrum is illustrated by the *ABL* proto-oncogene, which encodes a protein tyrosine kinase that promotes cell death in response to DNA damage. *ABL* is converted to an oncogene when a chromosome breakage and rejoining event translocates the ABL gene, which is located on chromosome 9, to the BCR gene on chromosome 22. This translocation produces a novel fusion protein, BCR-ABL, which is a highly active, unregulated protein kinase.

In contrast the normal ABL protein, the BCR-ABL protein inhibits apoptosis after DNA damage, thereby allowing damaged cells to survive and proliferate.

Oncogenic mutations can also simply increase proto-oncogene expression. Two examples are *MYC* and *BCL2*, which encode a transcription factor that stimulates cell cycle progression and a protein that inhibits apoptosis, respectively. Occasionally, a translocation moves these proto-oncogenes to a chromosome region containing the immunoglobin genes. When this occurs in a pre-B lymphocyte, where the immunoglobin genes are highly transcribed, MYC and BCL2 are overexpressed. This, in turn, promotes uncontrolled cell proliferation in the case of MYC, or resistance to cell death in the case of BCL2.

Thus, some mutations cause activation or overexpression of proto-oncogenes, creating oncogenes with supraphysiological or new functions that in turn promote cell growth or inhibit differentiation or cell death.

Tumor suppressor genes. Tumor suppressors inhibit cell growth, promote differentiation or stimulate apoptosis. They also suppress genomic instability, allowing cells to sense or repair DNA damage. In contrast to the gain-of-function mutations that activate proto-oncogenes, oncogenic mutations in tumor suppressor genes generally delete or inactivate the gene (loss-of-function). In most cases, both gene copies must be inactivated before loss-of-function is obvious. Thus, oncogenic mutations in tumor suppressor genes tend to be recessive.

Tumor suppressors include growth inhibitors and their receptors and signal transducers, transcription factors, pro-apoptotic proteins, and proteins that sense or repair DNA damage. Inactivating mutations are often chromosome aberrations that delete large segments of DNA.

However, more subtle mutations (for example, point mutations) can also inactivate tumor suppressors.

The most widely studied, and possibly most important, tumor suppressor genes are *RB* and *TP53*, which encode the pRB and p53 proteins. These proteins are at the heart of two major tumor suppressor pathways, each comprised of many interacting proteins. They are critical for the control of cellular senescence and mutated in >80% of human cancers.

pRB is a nuclear protein that indirectly controls the transcription of many genes. pRb is phosphorylated by several protein kinases, most prominently cyclin-dependent kinases (CDKs). When underphosphorylated, as it is in non-dividing cells, pRb prevents the initiation of DNA synthesis. pRB is progressively phosphorylated by CDKs as cells progress through G1. Phosphorylated pRB is inactive and cannot prevent cell cycle progression. Growth factors and inhibitors promote or prevent cell proliferation ultimately by controlling pRB phosphorylation. Many cancer cells have deletions or inactivating mutations in both copies of *RB*, and thus fail to arrest growth in response to growth inhibitory signals. Some cancer cells lack pRB mutations, but harbor inactivating mutations in the p16 tumor suppressor or overexpress cyclins D or E. p16 inhibits the CDKs that phosphorylate pRB, while cyclins D and E stimulate these CDKs. Thus, most mammalian cancers harbor mutations in the pRB pathway such that pRB is either physically or functionally inactive.

Most mammalian cancers also harbor mutations in the p53 pathway, whose functions overlap and differ from those of the pRB pathway. p53 is a transcription factor that also halts cell cycle progression. p53 is phosphorylated and stabilized in response to DNA damage, whereupon it induces genes that halt progression into S phase and mitosis, stimulate repair or induce apoptosis. Among the genes induced by p53 is the CDK inhibitor p21, providing an

interaction between the p53 and pRB pathways. p53 plays a pivotal role in damage sensing and repair and is considered a guardian of the genome. p53 function is abrogated by deletion (loss-of-function), but also by point mutations, which alter its properties as a transcription factor (gain-of-function). Many cancer cells harbor mutations in p53. Such cells fail to properly repair damaged DNA, but also fail to die. Consequently, they develop genomic instability, which accelerates mutation accumulation. Cancer cells that lack mutations in p53 *per se* generally have mutations in regulators of p53 expression or function.

Epigenetic factors and the cellular microenvironment. Non-mutational (epigenetic) events can also play a critical role in the development of cancer.

Within cells, physiological modification of DNA, such as methylation of cytosine, can strongly influence gene expression. Methylated DNA is generally transcriptionally silent, largely because it is packaged into chromatin is very compact. Loss of normal methylation can cause inappropriate proto-oncogene expression; conversely, inappropriate methylation can silence tumor suppressor genes.

Chromatin compaction is regulated largely by the presence and modifications to histones and other DNA binding proteins. These proteins are stripped from the DNA, and must be faithfully replaced, each time a cell undergoes DNA replication or during repair. Errors in replacement, or changes in cell physiology that alter the expression or modification of chromatin compaction proteins, can also result in inappropriate proto-oncogene expression or silencing of tumor suppressor genes.

Outside the cell, the surrounding milieu or microenvironment can be a critical determinant of whether and how a cell harboring oncogenic mutations expresses itself. It has

been known for several decades that tumor cells may develop into a fully malignant tumor, a slow-growing relatively benign tumor, or no tumor at all, depending on the tissue into which it is transplanted. We now know that all cells sense their microenvironment through specific receptors, including cell surface receptors. Some of these receptors bind soluble growth factors and inhibitors, whereas others (integrins) bind extracellular matrix components. In some tumors, these receptors are mutated. Most frequently, however, tumor cells express an altered pattern of receptors and integrins.

Recent experiments show that by manipulating the cellular microenvironment, particularly the extracellular matrix, some tumor cells can be induced to lose their malignant properties, including their ability to form tumors in animals. Conversely, chronic disruption of the cellular microenvironment -- for example by cells that ectopically express a protease that degrades the extracellular matrix -- can promote the development of cancer. As discussed below, even apparently normal tissue contains cells that harbor potentially oncogenic mutations. Such cells are prevented from progressing to more malignant phenotypes by the normal microenvironment; disruption of the microenvironment, then, allows such cells to express their oncogenic potential. Thus, the microenvironment within a tissue can be a powerful tumor suppressive mechanism which, in many cases, is dominant over oncogenic mutations.

Cancer and aging.

The largest single risk factor for developing cancer is age. The incidence of cancer increases exponentially with age, although death from cancer (cancer mortality) may decline at very old age (discussed below). The inevitable age-dependent rise in cancer incidence is a feature of multicellular organisms that contain a substantial fraction of mitotic cells. Model

organisms such as Drosophila melanogaster (flies) and Caenorhabditis elegans (worms) are composed primarily of post-mitotic cells, and hence do not develop cancer. Mammals, on the other hand, contain tissues that have a large population of mitotic cells, many of which tend to develop cancers with increasing age. What are the known and suspected causes of the age-dependent susceptibility to cancer?

Mutations increase with age. There is little doubt that mutations are a critical cause of cancer. Virtually all tumors harbor mutations. In fact, most tumors harbor dozens of mutations, many of which are detectable as large genomic amplifications or losses. The copious genomic changes that are characteristic of most tumor cells reflect an end-stage in tumorigenesis -- the point at which cells have presumably acquired genomic instability. Hence, mutations are rampant and hence have enabled the tumor cell to overcome the substantial intrinsic (for example, cellular senescence) and extrinsic (for example, the cellular microenvironment) tumor suppressive mechanisms. Prior to this stage, however, cells accumulate mutations, at least some of which are potentially oncogenic, in an age-dependent manner. Thus, mitotic (but, interestingly, not post-mitotic) tissues have been shown to accumulate a variety of mutations with increasing age, as detected by a neutral mutation reporter gene integrated into the genome of transgenic mice. Likewise, p53 mutations have been shown to accumulate in apparently normal tissue, particularly in skin exposed to ultra-violet radiation. Finally, apparently normal human tissue has been shown to accumulate mutations, particularly loss of heterozygosity, which predisposes cells to loss of tumor suppressor genes. Thus, potentially oncogenic mutations accumulate with increasing age.

Aging tissue and cellular microenvironments. Given that the tissue microenvironment can exert a powerful suppressive effect on oncogenically mutated genomes, can mutation accumulation alone explain the exponential rise in cancer incidence with age? Probably not. The exponentiality of the rise in cancer with age has been explained by the accumulation of four to eight critical mutations. However, tumors typically harbor dozens of mutations, and, as noted above, cells with oncogenic mutations are present in normal tissue. Moreover, the difference between a non-aggressive relatively benign tumor and an aggressive metastatic tumor cannot be easily explained by the number of mutations alone.

One explanation for the age-dependent rise in cancer is that mutation accumulation synergizes with the cellular microenvironment provided by aged tissue. Many tissues show an age-dependent decline in tissue function and structure, the latter often obvious by simple histological inspection. The cause(s) of the changes in tissue structure are incompletely understood. One cause may be the accumulation of senescent cells, which, as discussed earlier, secrete enzymes and cytokines that disrupt normal tissue architecture. However, other factors -- for example, crosslinking of extracellular matrix molecules by non-enzymatic glycation, or changes in circulating hormone levels -- may also contribute to age-dependent changes in tissue structure and hence cellular microenvironment.

Results from cell culture and animal experiments support the idea that aged tissues are a more fertile environment than young tissues for the growth of cancer cells. There is also evidence that senescent cells can create a more favorable environment than presenescent cells for the growth of tumor cells. Thus, the functional and structural changes that occur in aging tissues are likely an epigenetic cause for the development of cancer.

Despite the increased incidence of cancer with age, cancer mortality tends to decline at very advanced ages. Some cancers tend to be more indolent (slower growing and less aggressive) when they develop in very old individuals, compared to middle aged individuals. The reasons for this are not well understood. As discussed earlier, angiogenesis may be less efficient in very old individuals. In addition, the hormonal milieu in very old individuals may be less conducive than that of middle aged individuals to tumor progression. Whatever the case, cancer poses a major limitation to the health and longevity of mammals, and appears to result from an accumulation of mutations, as well as age-dependent changes in tissue structure and function.

Tumor suppressor and longevity assurance genes. During the evolution of complex multicellular organisms, there was a need to evolve genes that protect organisms from developing cancer -- that is, tumor suppressor genes. As such, at least some tumor suppressor genes act as longevity assurance genes (LAGs) -- genes that function to assure the health and fitness of organisms during their reproductive life span. Among mammals, cancer incidence begins to rise at about the midpoint of the maximum life span, or after reproductive fitness declines. Thus, tumor suppressors are effective LAGs, postponing cancer in young organisms and during the peak of reproduction and declining in efficacy, or even acting with antagonistic pleiotropy, only after the reproductive fitness has declined.

Tumor suppressor genes encode a variety of proteins, as discussed above, and many of them function during development as well as in adults (for example, those that control fundamental features of the cell cycle, differentiation or apoptosis). Such genes cannot be considered LAGs *per se* because their functions are also critical for normal development.

However, other tumor suppressor genes -- for example, *TP53* -- are classic LAGs because they are dispensable for normal development, but critical for preventing cancer in young organisms.

At least among mammals, the rate of aging, rate of cancer development, and maximum life span are very tightly linked. Thus, mice live roughly 3 years and develop cancer largely after a year and a half or so, whereas humans live roughly 120 years and develop cancer largely after 50 years or so. Despite this remarkable species difference in the rates of cancer development and aging, the major tumor suppressor pathways -- those controlled by p53 and pRB -- are well-conserved between mice and humans. There are still many gaps in our knowledge about the molecular mechanisms that link cancer and aging.

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